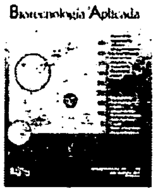

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Large-scale Production of recombinant Hepatitis B Surface Antigen from *Pichia pastoris*

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Introduction

An downstream process for the purification of HBsAg produced in *Pichia pastoris* was established first at laboratory scale [1] and further scaled-up from about 250- to 500-fold [2]. However, the effectiveness of the key steps for the large-scale production of *P. pastoris*-derived HBsAg had not been described yet.

We analyze here the performance of our production technology in terms of its ability to both render a highly pure HBsAg and remove most of the intrinsic (yeast total proteins, nucleic acids, carbohydrates, lipids) and extrinsic (immunopurification released immunoglobulin [Ig] G, endotoxin) contaminants. The results obtained verified that this technology satisfies most of the World Health Organization (WHO) requirements for the safe purification of yeast-derived, biologically-active HBsAg particles. Consequently, the vaccine (HEBERBIOVAC HB, Heberbiotec SA, Cuba), which is formulated with *P. pastoris*-derived HbsAg, has proven to be safe and efficacious, providing protection against hepatitis B infection [3]

Methods

Ten independent industrial batches of HBsAg, five made in 1993 and five in 1998, were obtained as previously described [2 and citations therein]. Briefly, the recombinant *P. pastoris* yeast strain was kept as a master seed lot at -70 °C to guarantee that each bioreaction run was started from the same original preparation. Under carefully controlled multiplication conditions, the HBsAg gene-containing yeast cells were passed from shake flasks into medium-scale bioreactors, and finally into a large-scale bioreaction unit. After harvesting, the yeast cells were disrupted to recover and purify HBsAg by a series of well-established steps [2 and citations therein] These included acid precipitation, adsorption/desorption from diatomaceous earth matrix and, finally, successive purification through immunoaffinity, ion-exchange and gel-filtration chromatographic procedures. Each step was currently checked to be within specifications of the WHO guidelines for quality and/or in-process control procedures approved by the National Control Authority. Also, the quality of the water and al buffer solutions were monitored for conductivity, pH and microbiological or pyrogenic contaminants, and were strictly controlled.

Results and Discussion

The ability of the *P. pastoris*-based technology for large-scale production of recombinant hepatitis B virus surface antigen (HBsAg) and to both reproducibly purify HBsAg and remove most of the relevant contaminants was ascertained by evaluating 10 industrial production batches: 5 in 1993 and 5 in 1998. At an early stage, the clarification of mechanically disrupted yeast cells by acid precipitation rendered HBsAg with a purity as low as 3.8 ± 0.6%. However, by adsorption/desorption from diatomaceous earth matrix, the purity of HBsAg rapidly increased to 18.8 ± 5%, which is suitable for chromatographic processing. This step also eliminated non-particulated forms of HBsAg, significantly lowered the amount of carbohydrates and lipids, and concentrated the HBsAg 4.8-fold. Finally, a sequential purification procedure that included large-scale immunoaffinity, ion-exchange, and size-exclusion chromatographies further purified the preparation, resulting in a product (HBsAg at a concentration of 1.3 ± 0.2 g/L) with a purity of 95% or more. Furthermore, each of the other contaminants measured reached the following low levels per 20 mg HBsAg: host deoxyribonucleic acid (less than 10 pg), carbohydrates (1.2 ± 0.02 mg), lipids (14 ± 0.28 mg), immunopurification-released IgG (less than 100 ppm), and endotoxins (106.7 ± 19.3 pg). These values were below those specified for recombinant DNA hepatitis B vaccines according to WHO guidelines.

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